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Another hijack! Some enteroviruses co-opt the c10orf76/PI4KB complex for their own good

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Enteroviruses, members of the Picornaviridae family, are non-enveloped and single-stranded RNA viruses responsible for several human diseases. During infection, these viruses build membrane-bound organelles, called replication organelles (ROs), where new virions are assembled. ROs are highly enriched in phosphatidylinositol 4-phosphate (PI4P) produced by the host lipid kinase PI4KB. In this issue of *EMBO Reports*, McPhail *et al* [1] characterize a complex, formed by PI4KB and the c10orf76 protein, which is involved in PI4P production. They show that this machinery is hijacked by specific enteroviruses such as coxsackievirus A10 for their replication.

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See also: **JA McPhail *et al*** (February 2020)

The Picornaviridae is a large family of RNA viruses including polioviruses, rhinoviruses, and coxsackieviruses, some of which are pathogenic to humans. A key step in the virus life cycle is the building of membrane-bound organelles, named replication organelles (ROs). These compartments grow at the expense of the host and serve as factories assembling new virions [2]. A striking feature of these virus-generated organelles is their unique lipid composition, which is highly enriched in phosphatidylinositol 4-phosphate (PI4P), a phosphoinositide predominantly found in the Golgi of non-infected cells (Fig 1) [3]. PI4KB (also known as PI4K IIIβ) is the main kinase producing the pool of PI4P in the Golgi membrane. The Golgi localization and the activity of PI4KB mainly depend on interactions with its partners, acyl-CoA-binding domain-containing protein 3 (ACBD3), which is required for the recruitment of the kinase at the Golgi surface, and the small guanosine triphosphate (GTP)ase adenosine diphosphate (ADP)-ribosylation factor 1 (Arf1), which activates PI4KB [2]. In infected cells, PI4KB accumulates on ROs fueling them with PI4P (Fig 1). In agreement with a crucial role for PI4P-rich membranes for enterovirus propagation, PI4KB inhibition drastically blocks viral replication [4].

To date, there is no evidence that viral factors directly recruit PI4KB on ROs. Instead, RO-localized viral 3A proteins co-opt cellular proteins such as the Golgi-resident protein ACBD3, which in turn leads to the redistribution of PI4KB at the RO surface [5]. 3A proteins also hijack other host proteins, such as the guanosine exchange factor (GEF) GBF1 (Golgi brefeldin A-resistant guanine nucleotide exchange factor 1), an Arf1 activator (Fig 1) [6]. The enigmatic c10orf76 protein (chromosome 10, open-reading frame 76, also known as Armadillo-like helical domain-containing protein 3—ARMH3) was previously identified in a synthetic lethality screen as a regulator of Golgi homeostasis and viral replication [7]. The c10orf76 protein binds PI4KB, and its depletion makes cells resistant to coxsackievirus A10. However, the molecular mechanism by which c10orf76 regulates PI4P levels and affects virus replication remained unknown. McPhail *et al* now characterize the c10orf76/PI4KB molecular complex and analyze its physiological function in regulating PI4P levels in the Golgi, and moreover, they document its role on enterovirus replication.

The authors first showed that the interaction between c10orf76 and PI4KB is direct. Using hydrogen–deuterium exchange mass spectrometry (HDX-MS) experiments, they identified the key residues involved in the binding of c10orf76 with PI4KB and engineered complex-disrupting mutants. They then used these mutants to study the subcellular localization and physiological role of the c10orf76/PI4KB complex. While PI4KB was recruited to the Golgi independently of its ability to interact with c10orf76, the reciprocal was not true. Indeed, while wild-type c10orf76 associated with the Golgi, the complex-disrupting c10orf76 mutant was diffusely distributed in the cytosol, indicating that c10orf76 needs to interact with PI4KB to be properly localized. In addition, the authors showed that c10orf76 controlled the recruitment of the Arf1-GEF GBF1 on the Golgi, thereby the enzymatic activity of PI4KB, and ultimately Golgi PI4P levels. This mechanism is consistent with a recent publication showing that c10orf76 interacts with GBF1 and controls GBF1 localization on the Golgi [8].

Finally, complementation assays in PI4KB-deficient cells showed that wild-type PI4KB successfully rescued replication of all viruses, while the complex-disrupting mutant form of PI4KB failed to restore coxsackievirus A10 replication. These data demonstrate the critical role of the c10orf76/PI4KB complex in the replication of specific viruses.

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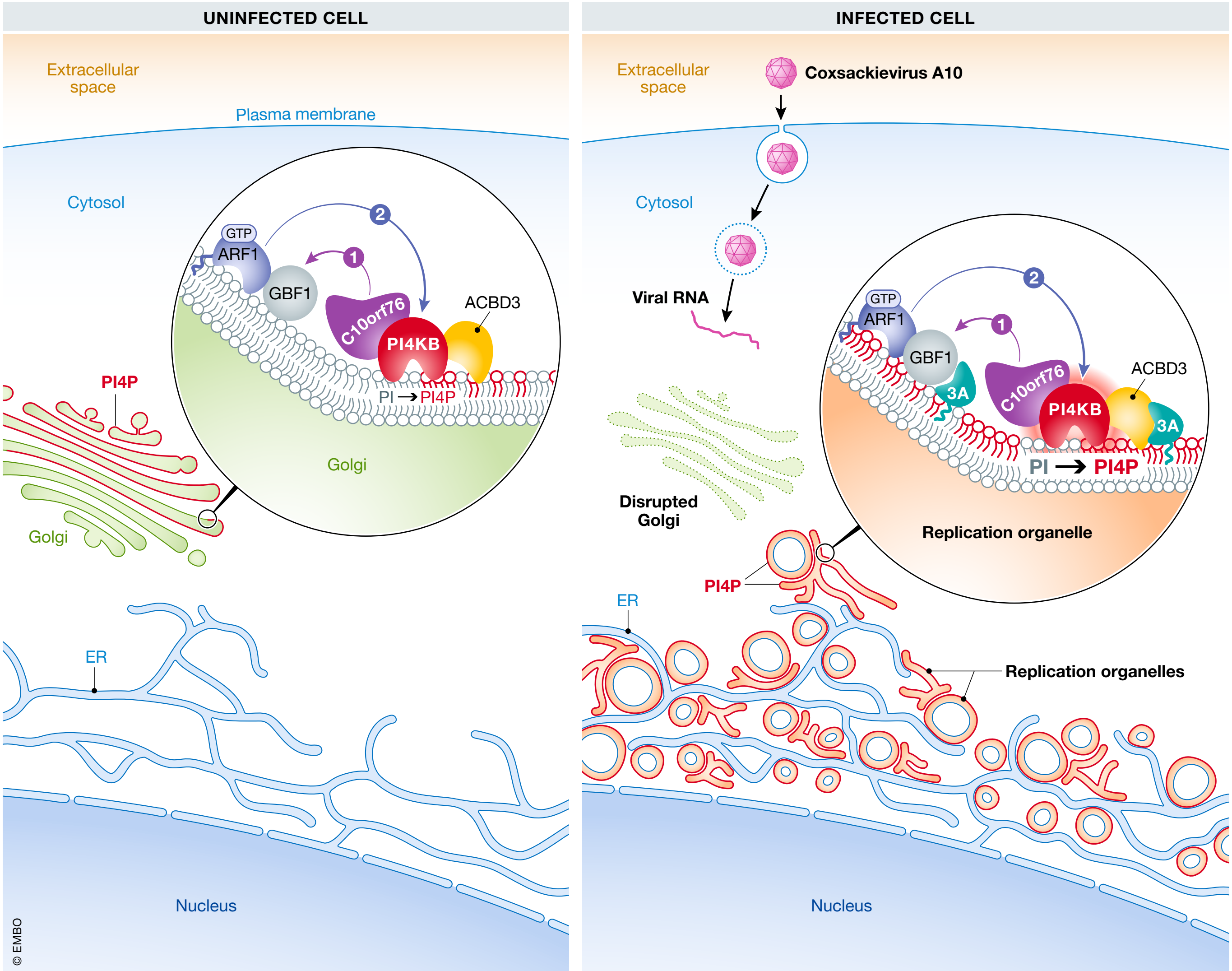

Figure 1. c10orf76 links PI4P metabolism and viral replication.

In coxsackievirus A10-infected cells, while the Golgi collapses, PI4P is highly enriched at the limiting membrane of specific organelles named replication organelles (ROs). In uninfected cells, the Golgi localization of PI4KB is controlled by its Golgi-localized protein partner ACBD3. c10orf76 directly interacts with PI4KB at the Golgi membrane and regulates the recruitment of GBF1 (arrow 1), a GEF and activator of the small GTPase Arf1. In its GTP-bound form, Arf1 activates PI4KB (arrow 2). In coxsackievirus A10-infected cells, this c10orf76/PI4KB-dependent PI4P synthesis machinery is hijacked at the surface of ROs. This hijacking is controlled by the RO-localized viral protein 3A, which mediates the recruitment of GBF1 and ACDB3, which in turn drives the recruitment of Arf1 and PI4KB.

The findings by McPhail *et al* reveal that the lipid kinase PI4KB recruits c10orf76 to the Golgi, which indirectly increases its activity and thus PI4P production. Furthermore, it is established that PI4KB is essential for enterovirus replication [4]. Therefore, one would expect a general involvement of the cellular c10orf76/PI4KB complex in enterovirus replication. It is thus intriguing that the c10orf76/PI4KB complex is only necessary for a subset of enteroviruses. The other members of this virus family must have evolved differently and hijack other cellular components able to activate PI4KB to obtain PI4P levels conducive to their replication.

The results suggest that the c10orf76/PI4KB complex acts at the limiting membrane of ROs to regulate PI4P metabolism in infected cells (Fig 1). The reason why enteroviruses hijack the PI4P synthesis machinery at RO membranes for their replication is not fully understood. One role of PI4P is to serve as a platform to recruit to ROs the viral and host components required for viral replication. Interestingly, PI4P is not the only lipid indispensable for enterovirus replication. Indeed, interfering with cholesterol homeostasis also inhibits enterovirus replication [2].

It is worth mentioning that ROs are not structures that float independently in the cytosol. Instead, they are intermingled with the other organelles of the host cell which they physically contact [2]. As such, ROs form extensive membrane contact sites (MCSs) with the endoplasmic reticulum (ER). MCSs are subcellular structures where the membranes of two organelles are physically connected without fusing. Molecularly, these domains are built by protein–protein and/or protein–membrane interactions [9]. Notably, PI4P is involved in the formation of ER-RO contact sites. For instance, PI4P recruits the lipid transfer protein OSBP (oxysterol-binding protein) to the RO surface [2]. Normally, OSBP connects the ER and the Golgi, by interacting on one side with the ER receptor proteins VAPs (vesicle-associated membrane protein (VAMP)-associated proteins), and on the other side with Golgi PI4P; in these contacts, OSBP counterexchanges cholesterol and PI4P. In enterovirus-infected cells, as a consequence of PI4P metabolism hijacking, OSBP is recruited to the RO surface and is involved in ER-RO contact formation, where it allows the counterexchange of cholesterol/PI4P. This facilitates the transport of cholesterol toward the membrane of ROs. Interestingly, pharmacological inhibition of OSBP decreases enterovirus replication, thus showing that these viruses require cholesterol transport toward ROs for virion assembly [10]. Hence, enteroviruses seem to hijack PI4P metabolism in order to remodel the spatial organization of organelles, and in particular to generate ER-RO contacts, which allow them to control the lipid composition of ROs. It is possible that within ER-RO contacts, other molecules and lipids are also transported. Future studies will help unravel the full extent of MCS function and how viruses use these structures to alter lipids, ions, and cellular signaling for their own good. This will potentially provide new therapeutic targets against enteroviruses.

References

1. McPhail J, Lyoo H, Pemberton J *et al* (2019) *EMBO Rep* 21: e48441
2. van der Schaar HM, Dorobantu CM, Albulescu L *et al* (2016) *Trends Microbiol* 24: 535 – 546
3. Altan-Bonnet N, Balla T (2012) *Trends Biochem Sci* 37: 293 – 302
4. Hsu N-Y, Ilnytska O, Belov G *et al* (2010) *Cell* 141: 799 – 811
5. Lyoo H, van der Schaar HM, Dorobantu CM *et al* (2019) *mBio* 10: e02742 – 18
6. Lanke KHW, van der Schaar HM, Belov GA *et al* (2009) *J Virol* 83: 11940 – 11949
7. Blomen VA, Májek P, Jae LT *et al* (2015) *Science* 350: 1092 – 1096
8. Chan CJ, Le R, Burns K *et al* (2019) *Mol Cell Proteomics* 18: 2285 – 2297
9. Balla T, Kim YJ, Alvarez-Prats A *et al* (2019) *Annu Rev Cell Dev Biol* 35: 85 – 109
10. Strating JRPM, van der Linden L, Albulescu L *et al* (2015) *Cell Rep* 10: 600 – 615